bio-protocol Preprint

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Puromycin Immunofluorescence

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An abbreviated version of this protocol was published in eLIFE in Aug 2020

Elongation inhibitors do not prevent the release of puromycylated nascent polypeptide chains from ribosomes

DOI: 10.7554/eLife.60048

Detailed protocol

Summary

This protocol for immunofluorescence is preceded by *in vitro* treatment of cells with puromycin followed by washout and paraformaldehyde fixation. Puromycin immunofluorescence allows quantification of the *amount* of nascent protein synthesis at a given point in time, but *DOES NOT* accurately report on the subcellular localization of nascent protein synthesis. See linked publication:

Elongation inhibitors do not prevent the release of puromycylated nascent polypeptide chains from ribosomes

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Notes

For best imaging quality, cells should be cultured and fixed on a glass surface. This protocol assumes the cells are cultured in glass bottom well plates, but similar results can be achieved with glass coverslips inside standard plastic plates. Ideally, cells should processed for immunofluorescence staining immediately after fixation. However, fixed cells can be stored in 1x TBS at 4°C for <24 hours without significant loss in quality of immunofluorescence staining. The protocol uses an anti-ribosomal P antibody to provide total ribosome immunofluorescence signal, which can be used to normalize the puromycin immunofluorescence signal on a per-cell basis. Any other anti-ribosomal antibody (e.g., anti S6RP, etc.) would also be suitable.

Materials

- Normal goat serum
- Tris-buffered saline (TBS)
- Tween 20
- Mouse anti-puromycin (clone 12D10, MilliporeSigma, catalog #MABE343, RRID:AB_2566826)
- Human anti-ribosomal P (Immunovision, catalog #HPO-0100)
- Goat anti-mouse Alexa647 (Invitrogen, catalog #A-21236, RRID:AB_141725)
- Donkey anti-human Cy3 (Jackson ImmunoResearch, catalog #709-165-149, RRID:AB_2340535)
- DAPI Fluoromount G (Southern Biotech, catalog #0100–20)

Procedures

1. Wash out residual paraformaldehyde (PFA) by several washes with excess TBS.
2. To permeabilize and block cells, incubate in 1X TBS supplemented with 10% normal goat serum (NGS) and 0.1% Tween 20 for 60 minutes at room temperature.
3. Remove blocking/permeabilization solution and incubate cells in 1x TBS supplemented with 2% NGS, 0.1% Tween 20, and primary antibodies. Primary antibodies are used as follows: mouse anti-puromycin at 1:500, and human anti-ribosomal P at 1:3000. Incubate in primary antibody solution for 60 min at room temperature.
4. Remove primary antibody solution and wash three times with 1X TBS (5-10 minutes each time).
5. Remove TBS and incubate cells in 1x TBS supplemented with 2% NGS, 0.1% Tween 20, and secondary antibodies. Secondary antibodies are used as follows: goat anti-mouse Alexa647 at 1:1000 and donkey anti-human Cy3 at 1:500. Incubate in secondary antibody solution for 30 min at room temperature.
6. Remove secondary antibody incubation and wash three times with 1X TBS (5-10 minutes each time).
7. Remove TBS and replace with enough DAPI Fluoromount G to cover the cells. Cells can be stored in DAPI Fluoromount G for 24 hours at 4°C prior to imaging.
8. Acquire fluorescence images of cells using epifluorescence or confocal microscopy.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hobson, B. and Sims, P. (2022). Puromycin Immunofluorescence. Bio-protocol Preprint. bio-protocol.org/prep1935.

2. Hobson, B. D., Kong, L., Hartwick, E. W., Gonzalez, R. L. and Sims, P. A.(2020). Elongation inhibitors do not prevent the release of puromycylated nascent polypeptide chains from ribosomes. eLIFE. DOI: 10.7554/eLife.60048